Disruption of p53 Function Sensitizes Breast Cancer MCF-7 Cells to Cisplatin and Pentoxifylline

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Abstract

The possibility that appropriately designed chemotherapy could act selectively against p53-defective tumor cells was explored in MCF-7 human breast cancer cells. These cells were chosen because they have normal p53 function but are representative of a tumor cell type that does not readily undergo p53-dependent apoptosis. Two sublines (MCF-7/E6 and MCF-7/mu-p53) were established in which p53 function was disrupted by transfection with either the human papillomavirus type-16 E6 gene or a dominant-negative mutant p53 gene. p53 function in MCF-7/E6 and MCF-7/mu-p53 cells was defective relative to control cells in that there were no increases in p53 or p21Waf1/Cip1 protein levels and no G1 arrest following exposure to ionizing radiation. Survival assays showed that p53 disruption sensitized MCF-7 cells to cisplatin (CDDP) but not to several other DNA-damaging agents. CDDP sensitization was not limited to MCF-7 cells since p53 disruption in human colon carcinoma RKO cells also enhanced sensitivity to CDDP. Contrary to the other DNA-damaging agents tested, CDDP-induced DNA lesions are repaired extensively by nucleotide excision, and in agreement with a defect in this process, MCF-7/E6 and MCF-7/mu-p53 cells exhibited a reduced ability to repair a CDDP-damaged DNA lesion. In each case, the increased CDDP sensitivity of MCF-7 cells with disrupted p53 to defects in G1 checkpoint control, nucleotide excision repair, or both. The G1 checkpoint inhibitor pentoxifylline exhibited synergism with CDDP in killing MCF-7/E6 cells but did not affect sensitivity of the control cells. Moreover, pentoxifylline inhibited G2 checkpoint function to a greater extent in MCF-7/E6 than in the parental cells. These results suggested that, in the absence of p53 function, cells are more vulnerable to G2 checkpoint abrogators. Our results show that a combination of CDDP and pentoxifylline is capable of synergistic and preferential killing of p53-defective tumor cells that do not readily undergo apoptosis.

Introduction

Mammalian cells demonstrate complex cellular responses to DNA damage, including activation of genes involved in cell cycle arrest, DNA repair, and apoptosis (1–4). Cell cycle arrest following DNA damage is mediated by a series of negative feedback control systems called checkpoints (1–6). Checkpoints monitoring DNA damage operate in late G1 and G2 phases and possibly during S phase. Arrest at these cell cycle junctions allows an extended time for DNA repair to take place before progression through critical phases of the cell cycle: S and M phases.

A critical component of the G1 checkpoint is the p53 tumor suppressor (4–6), which is also the most frequently mutated gene in human cancer (7, 8). The p53 gene encodes a nuclear phosphoprotein which normally activates G1 cell cycle arrest in response to at least some forms of DNA damage (6, 9, 10). p53 thereby extends the time available for DNA repair before S phase entry and so perhaps functions as a “guardian of the genome” (11). This scenario suggests that p53-defective tumors should have increased susceptibility to some DNA-damaging anticancer drugs (1). p53 can, however, also activate an apoptotic response to DNA damage, especially in hematopoietic and lymphoid cells (10, 12–15), which often overrides the G1 checkpoint response. In cell types programmed for apoptosis, loss of p53 function decreases sensitivity to a wide variety of DNA-damaging agents (10, 12–18), while in cell types of some solid tumors not inherently programmed for apoptosis, a clear relationship between p53 gene status and radiosensitivity or chemosensitivity has been more difficult to establish (19, 20).

Our recent investigations into the function of the p53-regulated gene product, Gadd45, have revealed that this protein stimulates nucleotide excision repair in vitro (21). This and other results we have gathered suggested that cells lacking p53 function might be more sensitive to anticancer drugs that inflict DNA damage of the type repaired primarily through nucleotide excision. We explored this possibility in the breast carcinoma MCF-7 cell line. Although this line contains functionally intact wild-type p53 genes, it represents a cell type that does not readily undergo apoptosis following DNA damage (22). Successful transfection of p53 into MCF-7 cells has been reported as have some of the biological properties of the p53 transgene (23). p53 function in MCF-7 cells was disrupted by constitutive high level expression of the human papillomavirus type-16 E6 gene or a dominant-negative, mutant p53 gene transfectected into the cells (24).

Cells were treated with CDDP,3 which induces DNA lesions repaired primarily through nucleotide excision (25–27), MMS, whose DNA lesions are repaired through base excision (26, 27), or with the topoisomerase II inhibitors, ADR and VP16, or γ-irradiation which induce DNA strand breaks (9, 10). We also investigated the effects of PENT (28, 29) to determine whether cells lacking p53 function would be more sensitive to the effects of G2 checkpoint abrogators.

Our studies revealed that p53 disruption sensitized MCF-7 cells to CDDP and PENT and suggested that some p53-defective tumors might be vulnerable to: (a) anticancer drugs that induce DNA damage of the type repaired through nucleotide excision; and (b) G2 checkpoint abrogators.

Materials and Methods

Cell Culture and Stable Transfection. The breast carcinoma MCF-7 cell line and the human colon carcinoma RKO cell lines were cultured in RPMI 1640 (Mediatech, Washington, DC) containing 15% fetal bovine serum (Intergen, Purchase, NY), 2 mM L-glutamine, 50 units penicillin, and 50 μg/ml streptomycin.

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3 The abbreviations used are: CDDP, cisplatin; MMS, methylmethanesulfonate; ADR, Adriamycin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; mu, mutant; PENT, pentoxifylline; VP16, etoposide.

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Synchronization of MCF-7 cells in G1-S phase was performed using aphidicolin (1 µg/ml; 24 h). For stable transfection, 1 × 10⁵ cells were seeded in 10-cm diameter Petri dishes 24 h before transfection. The human papillomavirus type-16 E6 gene (24) or a dominant-negative mutant p53 (143 Ala to Val) cloned into a pcMV plasmid (graciously provided by Bert Vogelstein, Johns Hopkins University, Baltimore, MD), which also contained a neomycin gene, were introduced into cells by calcium phosphate precipitation as described previously (30). Approximately 14 days later, G418-resistant colonies were selected, and after expansion, colonies demonstrating high level expression of E6 mRNA or mutant p53 protein were analyzed for their inability to G1 arrest and accumulate p53 and p21cip1/waf1 following γ-irradiation. The generation and characterization of colon carcinoma RKO cells expressing E6 or mutant p53 transgenes has been described previously (24, 31).

Irradiation and Drug Treatment. Irradiation was performed using a 137Cs source delivering γ-rays at a dose rate of 3.46 Gy/min. ADR, VP16, MMS, or CDDP were obtained from the National Cancer Institute. Stock solutions of ADR and MMS were prepared in deionized water and stored at −20°C until use. VP16 was prepared in DMSO. CDDP and PENT (Sigma Chemical Co.) were dissolved in PBS. Unless stated, cells were irradiated at room temperature or treated with chemical agents for 1 h at 37°C. PENT was added for 24 h following CDDP exposure or γ-irradiation.

Survival Assays. Cytotoxicity was evaluated using clonogenic and MTT survival assays. For clonogenic survival, cells were plated in replicate 10-cm diameter Petri dishes for 24 h, rinsed with fresh medium, and then irradiated or treated with various chemical agents for 1 h. Cells were then washed twice with fresh medium and incubated for 10–14 days; the number of colonies formed (>50 cells) were counted after staining with Giemsa. The plating efficiency of the untreated cells was between 50 and 75%. PENT (2 mm; 24 h) reduced clonogenic survival of the MCF-7 cell lines by less than 15%, and this was taken into account when analyzing clonogenic survival of the cisplatin and PENT combination. For the MTT assay, 250 cells in 200 µl of complete medium were placed into each well of a 96-well plate. Twenty-four h later, CDDP was added and left in the culture for 6 days. At this time, viability was assessed by the ability of cells to convert the soluble salt of MTT into an insoluble formazan precipitate which was quantitated spectrophotometrically following solubilization in DMSO, as described previously (32).

Flow Cytometry. Samples were prepared for flow cytometry as described previously (16). Briefly, cells were fixed in ice-cold 70% ethanol, washed with PBS, treated with RNase (Sigma; 500 units/ml) at 37°C for 15 min, and stained with propidium iodide (Sigma; 50 µg/ml). Cell cycle analysis was performed using a Becton Dickinson fluorescence-activated cell analyzer. 15,000 cells were analyzed for each point, and quantitation of cell cycle distribution was performed using the SOBR model program provided by the manufacturer.

Cell Electrophoresis and Western Blot Analysis. Samples were lysed on ice for 30 min in 1% NP40 prepared in PBS that contained 10 µg/ml leupeptin, 10 µg/ml aprotonin, 2 µg (4-aminophenyl)benzenesulfonyl fluoride, 1 mM sodium o-vanadate, 10 mM sodium fluoride, and 5 mM sodium Pp. Soluble protein was then boiled for 5 min in a SDS-loading buffer and 100 µg of total cell protein loaded onto 15% SDS-polyacrylamide gels. Proteins were then transferred electrophoretically to Immobilon membranes (Millipore, Bedford, MA) and then blocked for 30 min in 5% nonfat milk at room temperature. Immunodetection of p53 protein was performed with a monoclonal antibody (Oncogene Science), and for p21cip1/waf1 determination, a polyclonal antibody (PharMingen) was used. Antibody reaction was revealed using enhanced chemiluminescence (Amersham Corp., Arlington Heights, IL).

Host Cell (CAT) Reactivation Assay. The nonreplicating pSV2CAT plasmid, encoding bacterial CAT, was treated with 200 µM cisplatin in 1 mM Tris-HCl (pH 7.8), 10 mM NaCl, and 1 mM EDTA for 10 min at room temperature. The MCF-7 cell lines were then transfected with 5 µg of the cisplatin-damaged or undamaged pSV2/CAT plasmid using lipofectamine reagent (GIBCO-BRL). As an internal control, 0.5 µg of rPSV β-galactosidase was included in the transfections. Transient CAT gene expression was assayed 72 h after transfections. Quantitation of [14C]chloramphenicol conversion was performed by counting on a Betascope Analyzer (Betagen). Values were normalized to the β-galactosidase internal standard, and values were expressed relative to the CAT activity obtained for the undamaged CAT plasmid in each cell line (33, 34).

Results

Human Papillomavirus Type-16 E6 and mu-p53 Transfected MCF-7 Cells with Impaired p53 Function. The human papillomavirus type-16 E6 gene product, which stimulates degradation of p53 through a ubiquitin pathway (24, 35), and a dominant-negative mutant p53 transgene were used as a means to inhibit p53 function in MCF-7 cells. These transgenes under the control of the CMV promoter were transfected into MCF-7 cells by calcium phosphate precipitation, and G418-resistant colonies were selected and then expanded for analysis. Four clones expressing high levels of E6 mRNA and six clones expressing high levels of mu-p53 protein exhibited p53 disruption, as indicated by impaired responses to γ-irradiation induced the basal levels, especially of p21cip1/waf1 (Fig. 1A). The reduced level of p21cip1/waf1 in exponentially growing MCF-7/E6 cells could stem from inactivation of the basal transcriptional activity of p53 (31); however, we cannot exclude the possibility that E6 could also stimulate p21cip1/waf1 degradation. Impairment of G1 arrest is shown in the panels labeled 6.3 Gy in Fig. 1B. The mitotic inhibitor nocodazole was included to determine the ability of the cells to arrest in G2 (16). The lack of effect of nocodazole on the cell cycle patterns

Fig. 1. Functional characterization of G1 and G2 checkpoints in exponentially growing MCF-7 cells transfected with control (CMY), E6+, or mu-p53-containing plasmids. A, immunoblot analysis of p53 and p21 levels in control exponentially growing (+) and γ-irradiated (−) cultures. Cells were assayed 4 h following 6.3 Gy. B, cell cycle distribution 16 h following addition of nocodazole (0.4 µM/ml) and exposure to 6.3 Gy of γ-rays, or both. The X-axis shows propidium iodide fluorescence intensity used to assess DNA content of the cells. The similarity of the cell cycle profiles following γ-irradiation with or without nocodazole indicates that E6 or mu-p53 did not prevent MCF-7 cells from arresting in G2. The difference between the cell cycle profiles of CMV-, E6+, or mutant-p53-transfected cells shows that E6 and mutant-p53 abrogated the G1 checkpoint response to γ-irradiation.
A, MCF-7 cells transfected with control-CMV or E6-containing plasmids were treated was also observed when MCF-7 cells were transfected with a mu-p53 2 (P < 0.01). The enhanced sensitivity of MCF-7/E6 cells to cisplatin dose modification factor for equivalent cell killing was approximately transfected cells (Fig. 2A). For example, at a survival level of 10% for viability was determined using the MTT assay as described in “Materials and Methods.”

Fig. 2. Effect of p53 disruption on the sensitivity of MCF-7 and RKO cells to cisplatin. A, MCF-7 cells transfected with control-CMV or E6-containing plasmids were treated with cisplatin for 1 h, and survival was measured in clonogenic assays. Values shown are the means of at least four data sets; bars, SD. B, cisplatin was added to MCF-7 or RKO cells transfected with control-CMV, E6-, or mu-p53 genes and left for 6 days before cell viability was determined using the MTT assay as described in “Materials and Methods.”

shows that G2 arrest occurs equally well in the control and E6 or mu-p53 transfected cells. Furthermore, these results illustrate that in the absence of p53 function cells are still capable of G2 arrest.

p53 Disruption Sensitizes MCF-7 and RKO Cells to Cisplatin. The sensitivity of MCF-7 and MCF-7/E6 cells to several types of DNA-damaging agents was assessed in clonogenic survival assays (Table 1). Of the agents tested, only cisplatin exhibited a deficiency in repair of cisplatin-induced DNA lesions. The assay consisted of damaging a CAT reporter plasmid with cisplatin in vitro, transfecting the damaged plasmid into host cells, and then measuring CAT activity relative to a control transfection with an undamaged plasmid. Host cells having greater DNA repair activity should reactivate the damaged plasmid to a greater extent than cells having reduced DNA repair activity (33, 34). The results of a representative host cell reactivation assay are shown in Fig. 3A, and data compiled from two to three independent experiments are shown in Fig. 3B. Using the host cell reactivation assay, we found that pCMV transfected MCF-7 cells were able to recover CAT activity from the CDDP-damaged plasmid to approximately 34% of that achievable with the undamaged plasmid (Fig. 3B). When the same CDDP-damaged CAT plasmid was transfected into MCF-7/E6 or MCF-7/mu-p53 cells, however, we only recovered approximately 14 and 23% of control CAT activity, respectively, indicating that MCF-7/mu-p53 and MCF-7/E6 cells were approximately 1.5- to 2.5-fold less competent at reactivating the CDDP-damaged CAT plasmid compared to the control cells. Therefore, the repair of CDDP-induced DNA lesions in the transfected CAT-plasmid is greater when p53 function remains intact. Furthermore, the differences in reactivation of the CDDP-damaged plasmid in these lines was similar to the dose modification factor for equivalent cell killing amongst the different MCF-7 lines (Fig. 2).

PENT Synergizes with Cisplatin to Kill MCF-7/E6 Cells. Loss of p53 function could impair the ability of MCF-7 cells to survive CDDP treatment because of loss of the G2 checkpoint response and/or because of reduced DNA repair capacity. Loss of p53 function might, however, be partially compensated by activation of the G2 checkpoint, which was found to be operational in MCF-7 with disrupted p53 (Fig. 1). The G2 checkpoint would delay entry of DNA-damaged cells into mitosis, thereby allowing more time for constitutive DNA repair processes to remove DNA lesions before the critical chromosome segregation process (1–5). Abrogation of G2 checkpoint function could, therefore, be especially deleterious in DNA-damaged cells with defective p53. We investigated this possibility using PENT, a

Table 1 Effects of γ-rays, ADR, VP16, CDDP, and MMS on clonogenic survival of MCF-7/CMV and MCF-7/E6 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ID50</th>
<th>ID50</th>
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<tbody>
<tr>
<td></td>
<td>MCF-7/CMV</td>
<td>MCF-7/E6</td>
</tr>
<tr>
<td>γ-RAYS (Gy)</td>
<td>3.5 ± 0.8</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>ADR (μM)</td>
<td>1.88 ± 0.2</td>
<td>1.98 ± 0.4</td>
</tr>
<tr>
<td>VP16 (μM)</td>
<td>4.7 ± 0.1</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>CDDP (μM)</td>
<td>3.38 ± 0.2</td>
<td>1.69 ± 0.3</td>
</tr>
<tr>
<td>MMS (μg/ml)</td>
<td>16.3 ± 0.2</td>
<td>16.3 ± 0.1</td>
</tr>
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aID50, bP < 0.01.
Fig. 3. Effect of E6 and mu-p53 on the ability of MCF-7 cells to reactivate a CDDP-damaged CAT-plasmid. The CAT-plasmid was either untreated (—) or treated for 10 min with 200 μM cisplatin (+) before transfection into MCF-7/CMV, MCF-7/E6, or MCF-7/mu-p53 cells. Seventy-two h following transfection, the cells were lysed, and CAT activity was measured on a Betascope as described in “Materials and Methods.” CAT values shown are the relative conversion of chloramphenicol (Cm) to the acetylated species (Ac-Cm) relative to an undamaged plasmid in each cell line. All values shown were normalized to a β-galactosidase internal control transfected along with the CAT-plasmid. A, results from a representative experiment in which duplicate samples were taken; B, compiled data showing the mean and SD of CAT activity from two to three independent experiments.

### Table 2

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>% Control Activity (mean ± SD, n=7)</th>
<th>Relative to MCF-7/CMV</th>
</tr>
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<tr>
<td>MCF-7/CMV</td>
<td>54.1 ± 5.52</td>
<td>1.0</td>
</tr>
<tr>
<td>MCF-7/E6</td>
<td>13.8 ± 4.09</td>
<td>0.4</td>
</tr>
<tr>
<td>MCF-7/mu-p53</td>
<td>22.5 ± 1.91</td>
<td>0.66</td>
</tr>
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n = Sample Numbers

**Caffeine-derivative known to interfere with G2 checkpoint control (28, 29). Efforts were focused on the interaction of PENT with cisplatin in the MCF-7/E6 line, which had already been found to be more sensitive to cisplatin compared to control transfected cells (Table 1; Fig. 2).**

Clonogenic survival was measured for MCF-7 and MCF-7/E6 cells treated with cisplatin for 1 h and then incubated in PENT for 24 h. Fig. 4 shows the results of our analysis. PENT did not affect the survival of control MCF-7/CMV cells but strikingly enhanced CDDP-induced cell killing of MCF-7/E6 cells. PENT reduced the surviving fraction of MCF-7/E6 cells treated with 5 and 10 μM CDDP by approximately 2.3- and 30-fold, respectively (P < 0.01). PENT, therefore, also amplified the difference in CDDP sensitivity between MCF-7/CMV and MCF-7/E6 cells; at 5 μM CDDP, PENT amplified a 2.4-fold difference into a 5.5-fold difference, and at 10 μM cisplatin, PENT amplified a 7-fold difference into a 200-fold difference. The dose modification factor for equivalent cell killing in MCF-7 and MCF-7/E6 cells for the combination of CDDP and PENT was approximately 3.

**G2 Checkpoint Abrogation by PENT May Depend on p53 Function.** The effect of PENT on the G2 checkpoint of MCF-7/CMV and MCF-7/E6 cells was evaluated using ionizing radiation as a means of introducing DNA damage with well-defined timing (Fig. 5). The effect of PENT was first examined in random-phase exponential cultures (Fig. 5A) in an experiment that was otherwise similar to that shown in Fig. 1. The results suggest that PENT interferes with the radiation-induced G2 arrest in MCF-7/E6 cells but not appreciably in MCF-7/CMV cells. This can be observed by comparing the G2-M population in the panels marked 6.3 Gy with the G2-M population in the panels marked 6.3 Gy + PENT (Fig. 5A).

In order to more clearly evaluate the effects of PENT on the G2 checkpoint of MCF-7/CMV and MCF-7/E6 cells, a similar experiment was performed using cells that were synchronized in G1-S with the DNA polymerase inhibitor, aphidicolin (Fig. 5B). Nocodazole controls (Fig. 5B, first panel) show that washing the cells free of aphidicolin released the cells from the G1-S checkpoint and allowed them to progress and arrest in mitosis. Without nocodazole present, the majority of cells entered G1 of the next cycle (Fig. 5B, second panel). Cells that were irradiated (panels marked 6.3 Gy) became arrested primarily in G2 phase. There was little opportunity to arrest in G1, because aphidicolin blocks cells past the G1 checkpoint.4 The appearance of some irradiated MCF-7/E6 cells in G1 at 24 h suggests that

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4. P. M. O'Connor, unpublished observations.
In a controlled dose over a well-defined brief period of time and the protein (24, 35) or a dominant-negative mutant p53 gene. p53 function in cells, we transfected the cells with the human papillomavirus type-16 cells exposed to 6.3 y-rays. A, exponentially growing cells were irradiated with 6.3 y-rays normal p53 genes (22). In order to disrupt p53 function in MCF-7 readily undergo apoptosis following DNA damage, despite having programmed for apoptosis. The breast cancer MCF-7 and colon can function on the sensitivity of human tumor cell lines not inherently reduced effect when p53 function is intact.

marked 6.3 Gy + PENT). PENT had much less effect on irradiated allowed these cells to reenter G1 (compare 6.3 Gy panel with panel marked 6.3 Gy + PENT). PENT had much less effect on irradiated MCF-7/CMV cells. We infer from these results that PENT can inhibit the G2 checkpoint when p53 function is deficient but that it has a reduced effect when p53 function is intact.

Discussion

In the present study, we investigated the effect of disrupting p53 function on the sensitivity of human tumor cell lines not inherently programmed for apoptosis. The breast cancer MCF-7 and colon cancer RKO cell lines were suitable for this purpose, because they do not readily undergo apoptosis following DNA damage, despite having normal p53 genes (22). In order to disrupt p53 function in MCF-7 cells, we transfected the cells with the human papillomavirus type-16 E6 gene, the product of which stimulates destruction of the p53 protein (24, 35) or a dominant-negative mutant p53 gene. p53 function was tested by means of ionizing radiation which can be administered in a controlled dose over a well-defined brief period of time and the effects of which have been extensively characterized. We showed that the E6 and mu-p53 transfected cells were defective with respect to the normal p53 responses to ionizing radiation: an increase in p53 protein level, an induction of p21 ^WAF1/Cip1, and arrest in G1 phase of the cell cycle (Fig. 1).

Several types of DNA-damaging agents (γ-rays, ADR, VP16, CDDP, and MMS) were tested for differential killing of MCF-7/E6 cells relative to control transfected cells using clonogenic survival assays. Of these agents, only cisplatin exhibited a significant differential killing (Table 1; Fig. 2). Indeed, 90% of the E6-transfected cells were killed at a dose approximately 2-fold lower than that required to kill the same proportion of control MCF-7/CMV cells (Fig. 2). Similar results were obtained for MCF-7 cells expressing a mu-p53 transgene, indicating that the enhanced sensitivity was not a unique property of the E6 gene product. Furthermore, CDDP sensitization was not limited to MCF-7 cells since p53 disruption in human colon carcinoma RKO cells (present study) and the CDDP-resistant wild-type p53 ovarian cancer cell line A2780/cp70 (36) also enhanced sensitivity to cisplatin.

The lack of dependence of radioresistance on p53 function in MCF-7 cells (Table 1) is in agreement with previous studies in colon carcinoma RKO cells and murine fibroblasts (19) and studies in head and neck cancer cell lines (20) but contrary to studies in some other cell types (10, 12–18). These differences appear related to the apoptotic potential of the cell lines used in these different studies, although other possibilities are emerging (37). Taken together these studies highlight the importance of cellular context to the evaluation of the biological properties of the p53 tumor suppressor.

Cisplatin differs from the other agents tested in our study in that it causes DNA damage that is extensively repaired through nucleotide excision (25–27). The possibility that nucleotide excision repair has a p53-dependent aspect was suggested in recent studies on the role of the Gadd45 protein in the p53 pathway (21) and in a recent report that described sensitization of colon carcinoma RKO cells to UV light following p53 disruption (31). In order to test directly for an effect of p53 function on the ability of cells to repair CDDP-induced DNA damage, we damaged a reporter plasmid with CDDP and measured the ability of host cells to reactivate the transfected reporter gene (Fig. 3). We found that expression of E6 or mu-p53 indeed reduced the ability of cells to reactivate this reactivation. The result agreed qualitatively and quantitatively with the previously reported effect of p53 function in aiding the repair of a UV-damaged reporter plasmid in RKO cells (31). Thus, p53 appears to play a role in the repair of DNA damaged by cisplatin as well as by UV light. The p53 regulated gene product Gadd45 appears to be one component of the p53 pathway contributing to this DNA repair system (21), although additional components are also likely (38). At the present time, we cannot exclude the possibility that p53 disruption affects other cellular processes that contribute to altered cisplatin sensitivity. For example, transfection of MCF-7 cells with a mu-p53 transgene has recently been reported to suppress Bcl2 levels (23). We are, therefore, continuing to evaluate these cell lines.

Cell cycle checkpoints in G1 and G2 phases protect DNA-damaged cells by delaying entry into S phase and into mitosis, respectively. Since these checkpoints provide more time for DNA repair, we hypothesized that when the p53-dependent G1 checkpoint is inactivated, the protective role of the G2 checkpoint should become increasingly important. We tested this hypothesis by inhibiting G2 checkpoint function with PENT and expected to find that the combination of CDDP and PENT would synergistically and selectively kill MCF-7 cells with disrupted p53. We found this to be the case. We also found that PENT inhibited the G2 checkpoint more effectively in E6-transfected cells than in control cells. This was observed in assays of

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Fig. 5. Effect of PENT on G1 checkpoint function of MCF-7/CMV and MCF-7/E6 cells exposed to 6.3 y-rays. A, exponentially growing cells were irradiated with 6.3 y-rays and then incubated in the presence or absence of PENT (2 mM) for 16 h. Also shown is the effect of nocodazole (0.4 μg/ml) given for 16 h to unirradiated cultures. B, cells were synchronized in G1-S with aphidicolin (1 μg/ml; 24 h). Upon release, the cells were and then incubated in the presence or absence of PENT (2 mM) for 16 h. Also shown is the effect of nocodazole (0.4 μg/ml) given for 16 h to unirradiated cultures. B, cells were synchronized in G1-S with aphidicolin (1 μg/ml; 24 h). Upon release, the cells were and then incubated in the presence or absence of PENT (2 mM) for 16 h.
random phase cultures or in cells previously synchronized in G1-S with aphidicolin (Fig. 5). Similar observations have been made with caffeine by Powell et al. (39) and Russell et al. (40) and, taken together, indicate that cells with defective p53 are more susceptible to G2 checkpoint inhibitors. The molecular basis for enhanced G2 checkpoint abrogation in cells with disrupted p53 remains to be determined but could involve an altered regulation of the cdc2 kinase (29, 41) in cells with intact versus disrupted p53. If this is so, it would point to an involvement of the p53 tumor suppressor in G2 checkpoint regulation.

Although PENT is undergoing clinical trials following observations of PENT on the G2 checkpoint of cells with normal versus defective p53-defective cells by inhibiting the G2 checkpoint in such cells. We present evidence that PENT enhances the selectivity of CDDP against checkpoint response to DNA damage; and/or (b) impairing nucleotide function sensitizes human breast cancer MCF-7 and colon cancer means of exploiting differences in G2 checkpoint control between normal and tumor cells with defective p53 (1).

In summary, we have obtained evidence that disruption of p53 function sensitizes human breast cancer MCF-7 and colon RKO cells to CDDP and may do so by: (a) preventing the G1 checkpoint response to DNA damage; and/or (b) impairing nucleotide excision repair of CDDP-induced DNA lesions. In addition, we present evidence that PENT enhances the selectivity of CDDP against p53-defective cells by inhibiting the G2 checkpoint in such cells. We are presently exploring the molecular basis for the differential effects of PENT on the G2 checkpoint of cells with normal versus defective p53 with a focus on cdc2 kinase regulation. The involvement of the p53 tumor suppressor in cell cycle checkpoint regulation and DNA repair processes could be exploited in the development of new chemotherapy-stratagems for cancer treatment.

References

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